



The need for transboundary faunistics and conservation: first record of the Natterjack Toad (*Epidalea calamita*) in Czech Silesia, northeastern Czech Republic

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Abstract.—The Natterjack Toad (*Epidalea calamita*) has been severely declining in the northern and eastern parts of its range in past decades. An immense population decline has been recorded in the Czech Republic, the southeastern edge of the species range. Contrary to the majority of published distribution range maps of the Natterjack Toad, it is present only in the western part of the Czech Republic (Bohemia), scattered among mostly isolated populations. A new, relatively distant population was recently discovered in the northeastern part of the country, in Czech Silesia. The genetic analysis presented here demonstrates that the new population belongs to the evolutionary lineage that is widely distributed in the northeastern part of the species range. Thus, this population is not a possible exotic introduction, but probably represents a natural extension of Natterjack Toad populations from Poland to the south. We urge conservation actions to be taken immediately to support this unique population, which is presently inhabiting a dump site. We further emphasize the necessity of considering distribution records on both sides of state borderlines when faunistic research is conducted in borderlands.

Keywords. Amphibians, anthropogenic habitat, Bufonidae, Central Europe, distribution, edge populations, geographic range limit, phylogeography, range extension

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Introduction

The Natterjack Toad, *Epidalea calamita* (Laurenti, 1768), Bufonidae, is native to southwestern through northern Europe, and parts of eastern Europe (from Portugal to southern Sweden, Estonia, Belarus, and northwestern Ukraine), where it typically occupies sunny open sandy areas and heathlands (Sillero et al. 2014; Sinsch 2009). The species also tolerates saline habitats to some extent, including sand dunes on sea shores, and a substantial part of its range corresponds to the oceanic climate. However, within the continental climate zone it is more commonly found in anthropogenic habitats, such as agricultural areas like vegetable crop fields (Zavadil et al. 2011) and quarries (Arnold 2002; Speybroeck et al. 2016), due to the lack of natural habitats, such as floodplains. In the northern and eastern parts of the range, including Central Europe, the abundance of the Natterjack Toads has drastically declined in past decades (Dufresnes 2019).

In the Czech Republic (= Czechia), the Natterjack Toad is one of three bufonids and the rarest and most threatened anuran species, being registered as Critically Endangered (Jeřábková et al. 2017, 2019; Šandera et al. 2017). It is present only in Bohemia, the western part of the Czech Republic (Jeřábková and Zavadil 2020; Moravec 2019; Sinsch 2009; Vitáček and Zavadil 1994; Zavadil 1994, 1996), contrary to the majority of published species range maps in books and conservation/research resources (e.g., Beja et al. 2009; Sillero et al. 2014), which incorrectly show the range across the whole Czech Republic. The distribution in Czechia represents the southeastern edge of the species range (Beja et al. 2009; Sillero et al. 2014; Sinsch 2009). The distribution in Czechia is very scattered, with most of the populations isolated from each other (Fig. 1; AOPK ČR 2019; Jeřábková and Zavadil 2020; Maštera et al. 2015; Šandera et al. 2017), and on the edge of survival (Jeřábková et al. 2017, 2019; Šandera et al. 2017). A relatively continuous distribution in Czechia is present only in the Cheb and Sokolov Basins (Jeřábková

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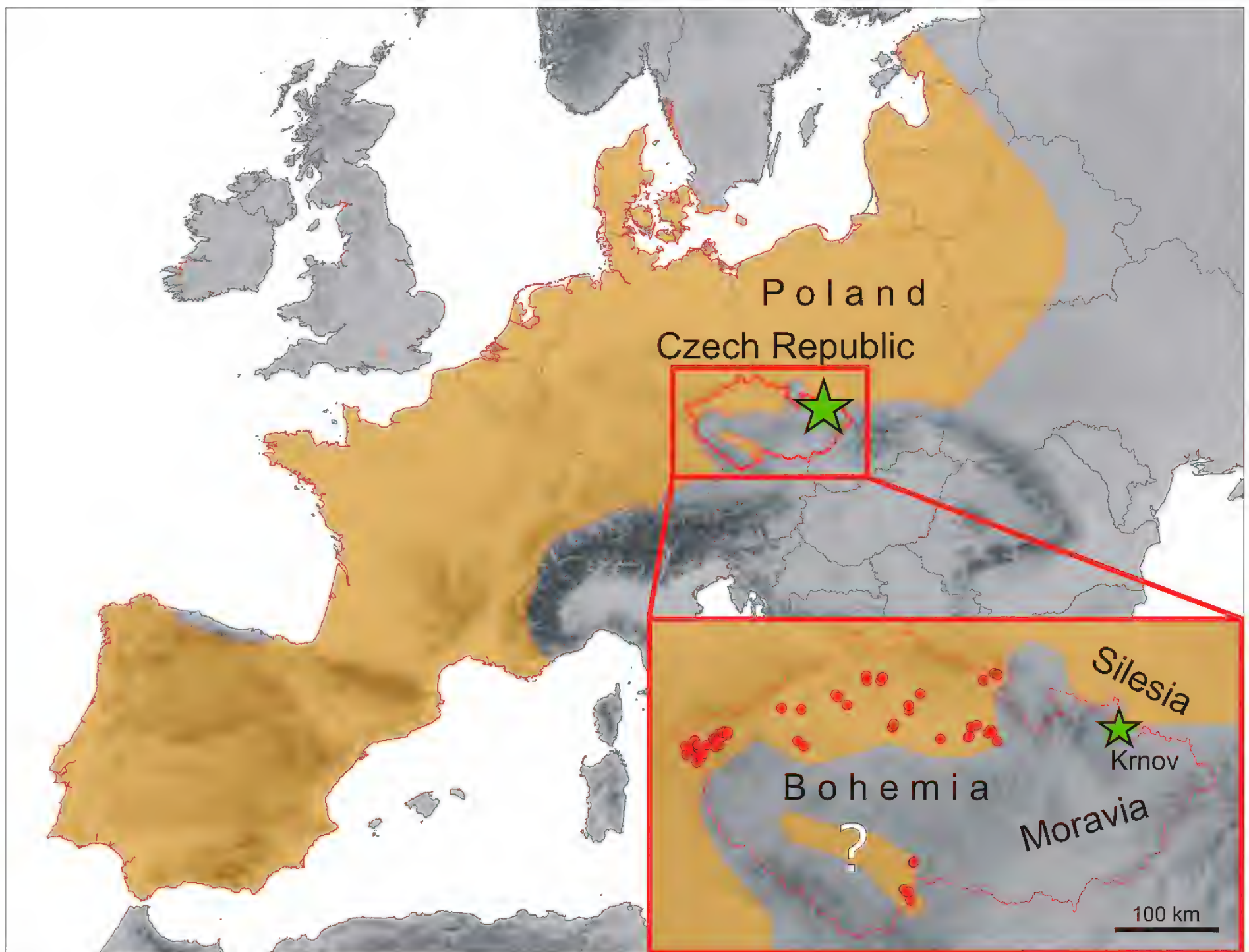


Fig. 1. Distribution range of the Natterjack Toad (*Epidalea calamita*) according to the *IUCN Red List of Threatened Species* (Beja et al. 2009), updated for the Czech Republic (inset). Red dots show all recent confirmed records (1997–2016; Šandera et al. 2017), demonstrating the very scattered distribution in the Czech Republic. The question mark denotes the region where the Natterjack Toad had occurred before 1990, but has since disappeared (AOPK ČR 2019; Jeřábková and Zavadil 2020). The green star shows the newly discovered population in Krnov, Czech Silesia.

and Zavadil 2020; Zavadil et al. 2011). The Natterjack Toad has never been reported reliably from Moravia or Czech Silesia, the eastern and northeastern parts of the Czech Republic (Šandera et al. 2017; Zavadil 1994, 1996). However, the recently published distribution map of the Natterjack Toad in Poland (Profus and Sura 2018) documents its occurrence in Polish Silesia (southern Poland), which indicates the possible presence of the Natterjack Toad on the other side of the border in Czech Silesia.

The presence of *E. calamita* in Czech Silesia was recently confirmed in Krnov, in the borderlands with Poland, highlighting the importance of transboundary faunistic studies. The site is less than 10 km from the nearest Polish locality. In this study, the genetic identity of the newly discovered population was tested to determine whether it was introduced from an exotic site. Specifically, the test can determine if the population originated in southwestern Europe, which hosts evolutionary lineages that differ from the lineages located in Central Europe (Rowe et al. 2006; Zeisset and Beebee 2014). The new

locality is described, the historic and present threats are discussed, and a baseline is proposed for the conservation management of this newly discovered population.

Materials and Methods

Study Area

The newly discovered locality, Krnov-Cvilín (50.0703°N, 17.7192°E, 390 m asl; Figs. 1–2), lies in the borderlands of the Czech Republic and Poland, in the foothills of the Jeseníky Mountains (northeastern part of the Bohemian Massif), and at the edge of the Silesian Lowlands. The area is a mosaic of forested and agricultural landscapes, which changes into a predominately agricultural landscape in the Silesian Lowlands.

Genetic Methods

Genomic DNA was extracted from toe clips of five Natterjack Toads using a spin column-based extraction



Fig. 2. Newly discovered locality of the Natterjack Toad (*Epidalea calamita*) in Krnov, Czech Silesia, Czech Republic. The distribution site is located within the Cvilín demolition waste dump, which used to be a sand quarry. **(A)** The site is now completely filled-up by demolition waste, an unfavorable condition for several species of amphibians present at the site. **(B)** Puddles are formed in small depressions after the movement of heavy-weight vehicles. However, the formation of such small puddles will probably stop in the near future as the waste dump is now closed. This is another unfavorable condition for this population, together with the surrounding grounds being overgrown by dense vegetation.

kit and following the manufacturer’s manual. One sample was from a subadult specimen from the newly discovered population (Fig. 3) in the northeast of the Czech Republic (Krnov), while four samples for comparison were from the western Czech Republic (Odrava-Obilná, sand quarry; 50.1030°N, 12.4732°E, 425 m asl). PCR amplification and DNA sequencing targeted a fragment of 16S rRNA using the primers and protocol reported by Gvoždík et al. (2010). The nucleotide sequences obtained were supplemented by available conspecific 16S rRNA data from GenBank. The GenBank data were derived from 24 individuals from throughout the species distribution range, including one additional sample from the western Czech Republic (GenBank KF665137; Lomnice, Erika sand quarry; 50.2117°N, 12.6064°E, 480 m asl) and two outgroup taxa (*Bufo bufo* and *Bufo viridis*, both from Czechia). GenBank numbers and countries of origin are given in Fig. 4, and the new sequences were deposited in GenBank (MT396931–MT396935). Alignment of the nucleotide sequences was prepared using the Mafft algorithm (Kato and Standley 2013), with default settings as incorporated in Geneious R8.1 (Biomatters, Auckland, New Zealand), and included 548 aligned sites. The maximum-likelihood phylogenetic tree was constructed by the RAxML algorithm (Stamatakis 2014) using the general time-reversible model of substitution evolution with rate heterogeneity, and 100 bootstrap pseudoreplicates to assess the branching supports.

Results and Discussion

Discovery of the New Population and Site Characteristics

In July 2019, an amateur naturalist found several specimens of the Natterjack Toad in a demolition waste dump in Krnov-Cvilín. The toads were originally erroneously identified as the Green Toad (*Bufo*

viridis), and later re-identified according to photographs by the first author of this contribution. The finding was popularized in internet media as a finding of “the rarest Czech anuran in a dump” (Kuba 2019). On 8 August 2019 at 2110–2220 h, with an air temperature of 18–20 °C, six specimens (four adults, one older subadult, and one younger subadult) of *E. calamita* were found at the dump site in Krnov, Cvilín Quarter, which represented the first confirmed record of the Natterjack Toad in Czech Silesia (Fig. 3). Subsequent surveys in August and September 2019 brought more findings of around ten individuals of different ages, including both adults and subadults. Together with the finding of the younger subadult (Fig. 3D), the various ages of the individuals suggest that the population was then – or until recently had been – reproducing.

The distribution site in Krnov-Cvilín (Fig. 1) is approximately 2.3 km from the border with Poland, which is formed by the Opava River. Climatically, the site is in a warmer region of the Czech Republic (Quitt 1971), with an average temperature in January (coldest month) of -2.6 °C and in July (warmest month) of 17.0 °C (<https://en.climate-data.org/>). Geomorphologically, the site is within the Zlatohorská Highlands, a part of the Jeseníky Mountains. According to the Kartierung der Flora Mitteleuropas (KFME) mapping grid, a widely used floristic and faunistic mapping system in the Czech Republic, the Cvilín dump lies within grid cell #5972 (Pruner and Mika 1996).

Genetic Identity

The specimen from the Krnov population has the same haplotype as the specimens from Western Bohemia (Czechia), as well as specimens from northern France, the Netherlands, and Denmark (Fig. 4). Closely related haplotypes originated from Sweden. In the Western Bohemian site (Odrava-Obilná), one additional haplotype

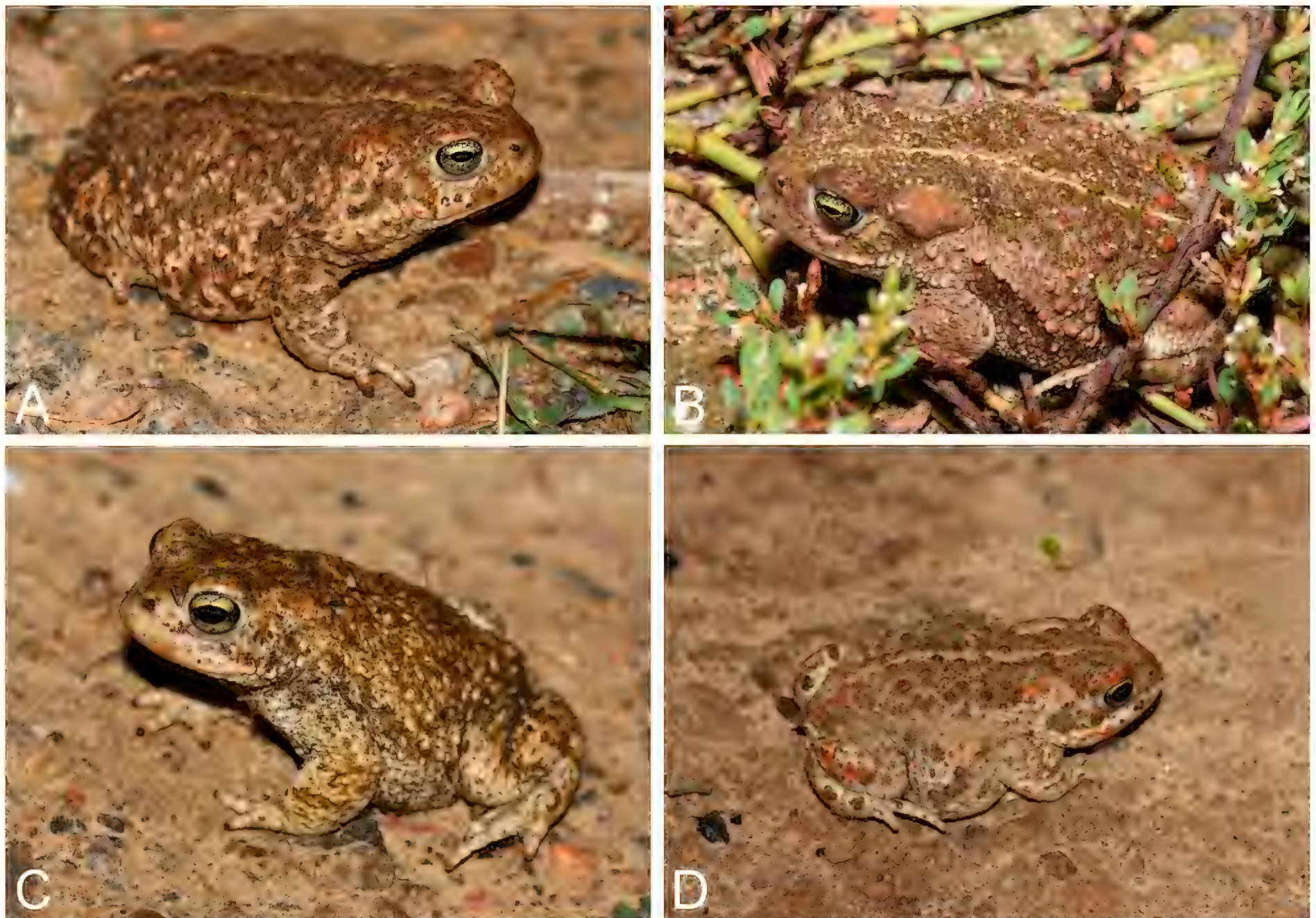


Fig. 3. Individuals of the Natterjack Toad (*Epidalea calamita*) from the newly discovered population in Krnov, Czech Silesia, Czech Republic, all found active at night in August 2019. (A) Adult female (SVL 74 mm), (B) genetically tested subadult specimen, (C) adult male with an indistinct dorsal stripe, and (D) the smallest subadult (SVL 34 mm) that was found.

was found. All of these haplotypes form a well-supported clade, which corresponds to Clade A according to Rowe et al. (2006). A comparison of the results of this study with those of Rowe et al. (2006) and Zeisset and Beebee (2014), indicates that Clade A is distributed from northern Spain and western and northern France to Britain, Ireland, the Netherlands, Denmark, Sweden, Estonia, and Central Europe, including Germany, Poland, and the Czech Republic. A higher genetic diversity in the Natterjack Toad is found only in the Iberian Peninsula, southern France, and partially in Switzerland and southwestern Germany (Fig. 4; Rowe et al. 2006). The remainder of the range, including the Czech Republic, is occupied by a genetically relatively uniform evolutionary lineage, which probably colonized this region during the post-glacial expansion (Rowe et al. 2006; Zeisset and Beebee 2014).

For effective conservation management, it is important to point out that the specimen from Krnov is a member of this widespread Central and Northern European lineage (Clade A *sensu* Rowe et al. 2006), which is also native in Czechia. This means that the Krnov population is not a distant, exotic introduction, specifically from southwestern Europe (e.g., southern Iberia or southern France). The most probable hypothesis for this population's occurrence in Krnov is an autochthonous

origin with biogeographic and evolutionary connections to populations in the Silesian Lowlands in Poland. However, the application of a population-genetic approach is needed to more precisely elucidate the eco-evolutionary relationships of the Krnov population to the nearest neighboring populations both to the north in Poland and to the west in Bohemia. This will be a crucial step for properly defining conservation units, which is an essential step for the conservation management of the Natterjack Toad in the Czech Republic.

Recent History of the Locality

The site is presently a demolition waste dump that is in the process of being closed because its capacity is now full (Fig. 2). According to the town chronicle (F. Kuba, September 2019, pers. comm.) and long-time residents' memories, the site was an active sand quarry before World War II. A small pond was present when the quarry was abandoned. At the end of the 1960s, ecological degradation began when liquid toxic waste was first deposited in the abandoned quarry, and this continued until 1983. Since 1985, the site has been "recultivated" in several serial attempts which were interrupted by the continuing usage of the site as a dump. The "recultivation" landfilling was done mainly using demolition waste and



Fig. 4. Maximum-likelihood phylogenetic tree showing the position of an individual from Krnov (in red) within the Clade A (*sensu* Rowe et al. 2006), indicating the non-exotic origin of the Krnov population. All other Czech samples (from Western Bohemia) are in bold. Codes correspond to GenBank numbers, and numbers at nodes are bootstrap support values

soil. The last remains of the pond had been visible until 1997, when it was completely covered by the landfilling. However, puddles (as potential breeding sites) were probably formed after rains. The last, final phase of landfilling began in 2007 and had been ongoing until October 2019, when the dump was formally closed due to its full capacity.

Present Situation and Threats

The present size of the Cvilín dump is approximately 100 × 250 m, and the main anthropogenic disturbance is the frequent usage of the site by heavy-weight vehicles, similar to the open-pit mines of Western Bohemia (Sokolov Basin) where the Natterjack Toad is still relatively common (Zavadil et al. 2011). The site is filled with rubble, other demolition waste, and soil, with the depth of the waste layer of about 5–10 m. Some

pioneer vegetation is growing on the soil surface, and the Natterjack Toads are commonly found active near the Knotgrass (*Polygonum arenastrum*) growth, where they hide when disturbed. The ridden, smooth soil surface allows for the formation of small puddles after heavy rains. Although highly probable, it is not clear whether they are sufficient for the reproduction of the Natterjack Toads. Within the site, there is presently a single small artificial pond made from a black plastic waterproof sheet (approximately 5 × 3.5 m, maximum depth ~0.3 m). This pond was built by the local municipality to provide a breeding habitat for the local amphibian population (without the previous knowledge of the Natterjack Toad's presence). However, this single small artificial pond is not large enough to provide a reproduction site for all the local amphibians. Moreover, it is unclear which species can utilize this pond for reproduction. Tadpoles of *Pelobates fuscus* and small postmetamorphic

juveniles of *Pelophylax* kl. *esculentus* were found in the pond in August 2019, suggesting that these two species are able to breed in this sole artificial pond of limited size and volume. In addition, *Bombina variegata*, *Bufo bufo*, and *Bufo viridis* were recorded within the Cvilín dump site, but it is not clear whether they successfully breed within the dump. [However, see the **Note** at the end of this article for updated information.] Krnov-Cvilín is thus one of the few sites in Czechia, where the three bufonids occur syntopically. The other amphibians known from the whole Krnov region are: *Hyla arborea*, *Pelophylax ridibundus*, *Rana arvalis*, *R. dalmatina*, *R. temporaria*, *Salamandra salamandra*, *Ichthyosaura alpestris*, *Lissotriton montandoni*, *L. vulgaris*, and *Triturus cristatus* (AOPK ČR 2019; Jeřábková and Zavadil 2020; Moravec 1994; Šiffner 2011).

Recommendations for Conservation Management

Conservation actions for the Natterjack Toad in the Cvilín dump are in preparation. Briefly, four initial recommendations are: (1) conserve the present pedologic conditions in the majority of the soil surface, but remove the demolition waste to uncover the original substratum in a part of the area; (2) retain water by building several shallow water reservoirs of different sizes and depths for reproduction, ideally on the original substratum (with up to 50 cm depth and slightly gradually sloping banks); (3) block secondary succession to avoid the overgrowth of dense and/or high vegetation; and (4) eliminate potential revegetation actions.

Importance of Transboundary Faunistics and Conservation

The newly discovered locality extends the known distribution area of *E. calamita* in the Czech Republic by 110 km (by air) east of the nearest registered location, which is a sand quarry near Plchovice-Smetana in Eastern Bohemia (50.0526°N, 16.1869°E; grid cell #5963; AOPK ČR 2019). Thus, the Natterjack Toad is newly listed as a species present in Czech Silesia. The newly discovered locality in Krnov is the easternmost known distribution site of the species in the Czech Republic, and forms the southeastern margin of the distribution range of the species in Central Europe. These individuals represent a unique Natterjack Toad population within the Czech Republic, geographically separated from the Bohemian populations, and therefore of particular conservation importance. However, it is possible that more populations are present in Czech Silesia or Northern Moravia which have yet to be discovered [see **Note** at the end of this article]. The Krnov population has a biogeographic affinity to the Polish Upper Silesian populations (see maps in Profus and Sura 2018, and the online atlas at <http://www.iop.krakow.pl/PlazyGady>). The nearest presently known distribution site is approximately 8

km to the northeast (behind the Opava River), in a sand quarry near Zubrzyce, Poland (50.1274°N, 17.7787°E, 290 m asl; grid cell #5872), a locality discovered only recently in 2018 (M. Pabijan, September 2019, pers. comm.). However, other Upper Silesian localities in Poland, in the borderlands with the Czech Republic, have been reported (Profus and Sura 2018; Świerad 1998). These earlier findings could serve as an indication of the possible presence of the Natterjack Toad in Czech Silesia, which would allow the discovery of the Krnov-Cvilín locality in a more favorable condition, before the original sand quarry was completely destroyed. Considering the distance between the Krnov population and the nearest previously known locality, we can assume that the two populations (and possibly other nearby populations) are probably genetically connected within a metapopulation network (Sinsch 2017).

This case highlights the importance and need for transboundary faunistics and conservation in general. All too often, local or national faunistics and conservation actions are conducted without proper knowledge of the situation that is right on the other side of the country borderline. Considering that species distributions in neighboring countries can bring new important discoveries (e.g., Najbar et al. 2011; Strachinis et al. 2019; Viček et al. 2010), we urge all those involved to consider the situations on both sides of borderlines when faunistic research and conservation actions are conducted in borderland areas.

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Note: In 2020, after the acceptance of this article, another population of the Natterjack Toad (*Epidalea calamita*) was discovered by the authors and Filip Šiffner near Osoblaha in Czech Silesia (50.2624°N, 17.6922°E, 270 m asl), 21 km to the north from the Krnov-Cvilín locality (by air). At the Krnov-Cvilín site, the Natterjack Toad successfully bred in a naturally-formed puddle in June–July, laying eggs after heavy rains, and larvae metamorphosed in approximately three weeks. The artificial pond served as a successful breeding site for *Bombina variegata*, a Critically Endangered species in the Czech Republic.